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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Chenicheri Hariharan Nair et al.

Serial No.: 10/029,014 Filed: December 21, 2001

Examiner: Not Yet Known Group Art Unit: 1741

For: RADIAL ELECTROPHORESIS APPARATUS AND METHOD

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail on this date: June 11, 2002 and is addressed to Commissioner for Patents, Washington, D.C. 20231

Frank M. Gasparo, Esq. (Reg. No. 44,700) June 11, 2002 Date

Commissioner for Patents
Washington, D.C. 20231

SUBMISSION OF PRIORITY DOCUMENT

SIR:

Applicants hereby submit a certified copy of the priority document: Australian Provisional Application No. PR 2223 filed on December 21, 2000 in the name of Gradipore Limited.

The Commissioner for Patents is hereby authorized to charge payment of all fees associated with this communication to Deposit Account No. 02-0393.

Respectfully submitted,

Date: June 11, 2002

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FMG:ik
Enclosure

AUSTRALIA

Patent Office
Canberra

I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR 2223 for a patent by GRADIPORE LIMITED filed on 21 December 2000.

WITNESS my hand this
Tenth day of December 2001

J R Yabsley

JONNE YABSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

AUSTRALIA

Patents Act 1990

Gradipore Limited

PROVISIONAL SPECIFICATION

Invention Title:

Electrophoresis device and method

The invention is described in the following statement:

Technical Field

The present invention relates to apparatus and method for electrophoretic separation of compounds.

Background Art

A variety of electrophoretic techniques have been developed for the processing of charged macromolecules with the most successful being a polyacrylamide gel electrophoresis, isoelectric focussing and capillary electrophoresis. Attempts to translate this resolution to a preparative scale has been less successful because the increasing volume of the porous matrix in a larger apparatus makes heat removal more difficult. Nevertheless, partial success has been achieved for some preparative systems including free flow electrophoresis, recycling isoelectric focussing, multi-compartment electrolyser, and conventional gel preparative systems. Although there are a variety of techniques for processing charged molecules, often the presence of salts or other compounds in the preparation can hinder the separation or, alternatively, high concentration of salts may be present in the end product.

Unfortunately, many of the techniques presently available result in loss of some of the macromolecules/compounds, inactivation of the macromolecules/compounds, or dilution of the macromolecule/compound preparation.

The present inventors have now developed a new apparatus which is adaptable for several different separation modes and can be used for large scale separations.

Disclosure of Invention

In a first aspect, the present invention provides an electrophoresis apparatus comprising:-

(a) an inner or central electrode positioned in an inner buffer stream;

(b) an outer electrode positioned in an outer buffer stream;

(c) at least two substantially non-planar membranes positioned generally radially between the inner and outer electrodes forming at least one sample stream.

Preferably, (c) is a plurality of non-planar membranes positioned generally radially between the inner and outer electrodes forming at least one sample stream and at least one separation stream.

Preferably, some of the membranes are electrophoresis separation membranes and others are restriction membranes. Preferably, the restriction membranes are positioned between the inner or outer buffer streams and the adjacent a separation or sample stream. One of the restriction membranes can be a charged membrane which prevents the substantial bulk movement of fluid under the influence of an electric field.

The electrophoresis separation membranes are preferably made from polyacrylamide and have a molecular mass cut-off of at least about 3 kDa. The molecular mass cut-off of the membrane will depend on the sample being processed, the other molecules in the sample mixture, and the type of separation carried out.

The restriction membranes are also preferably formed from polyacrylamide. The molecular mass cut-off of the restriction membrane will depend on the sample being processed, the other molecules in the sample mixture, and the type of separation carried out.

The charged membrane if used is preferably a cellulose tri-acetate membrane (CTM). It will be appreciated that the charged membrane can be formed from any other suitable membrane material such as polyvinyl alcohol (PVAl). The present inventors have found that a CTM having a nominal molecular mass cut-off of 5, 10 or 20 kDa is particularly suitable for use in the apparatus.

In one preferred embodiment, the electrodes are made of titanium mesh coated with platinum. As the outer electrode is generally non-planar and positioned generally radially to the inner or central electrode, any suitable formable material can be used for the electrode.

One or more of the membranes can also be formed to act as an isoelectric or amphoteric membrane containing a defined charge.

The temperature of the buffer and solutions in the sample and separation streams or reservoirs can be controlled by a suitable cooling/heating means. The apparatus may also be positioned in a controlled-temperature environment to maintain a desired temperature during removal of the salts.

The apparatus may have its own power supply or can be connected to an external power supply.

The membranes may be formed as a multilayer or sandwich arrangement. The thickness of the membranes can have an effect on the

separation or movement of compounds. It has been found that the thinner the membrane, faster and more efficient movement occurs.

The restriction membrane positioned between the inner buffer stream and the sample stream and between a separation stream adjacent the outer buffer stream can have the same molecular mass cut-off or different cut-offs therefore forming an asymmetrical arrangement.

Flow rates of buffer and of the sample through the buffer stream and separation stream can have an influence on the separation of compounds. Rates of milliliters per minute up to liters per minute can be used depending on the configuration of the apparatus and the sample to be separated.

The temperature of buffers and sample solutions in the apparatus can be controlled by a suitable cooling/heating means. The apparatus may also be positioned in a controlled-temperature environment to maintain a desired temperature during use.

The distance between the electrodes can have an effect on the separation or movement of compounds through the membranes. It has been found that the shorter the distance between the electrodes, the faster the electrophoretic movement of compounds.

Voltage and/or current applied can vary depending on the separation. Typically up to about 500 volts can used but choice of voltage will depend on the configuration of the apparatus, buffers and the sample to be separated or treated.

The membranes can be formed in any non-planar shape such as dish, u-shaped, cone, oval or circular. Preferably, the membranes are generally circular in cross-section positioned around the inner or central electrode. Thus, the apparatus is in the form of a tube arrangement positioned around the inner or central electrode. In this form, the outer electrode is also generally circular in cross-section such that when an electric field is applied between the electrodes, charged molecules can be caused to move through a membrane in 360^0 direction.

In one preferred from, at least one stream positioned between the two electrodes can be rotated axially providing a centrifugal force to material in the stream. In this form, it is possible to separate materials by electrophoresis and centrifugation.

An advantage of the apparatus according to the present invention is that the surface area of the membranes is significantly larger than an

apparatus with a series of planar membranes where the electromotive force applied being in one direction.

The apparatus may also further include buffer reservoir(s) for passing buffer to the inner and outer buffer streams, sample and separation reservoirs for passing sample and collecting separated components to and from the respective sample and separation streams.

In a second aspect, the present invention provides a method of separating a compound by electrophoresis, the method comprising

(a) placing a sample containing a compound to be separated in a sample stream of a electrophoresis apparatus comprising an inner or central electrode positioned in an inner buffer stream; an outer electrode positioned in an outer buffer stream; a plurality of non-planar membranes positioned generally radially between the inner and outer electrodes forming at least one sample stream and at least one separation stream;

(b) adding buffer to the inner and outer buffer streams;

(c) adding buffer or solvent to the separation stream;

(d) applying an electric potential between the electrodes causing at least one compound in the sample stream to move through a membrane into the separation stream; and optionally

(e) collecting the compound from the separation stream.

Preferably, the method involves use of the electrophoresis apparatus according to the first aspect of the present invention.

The sample may be any sample and the compound any material capable of being caused to move through a membrane under the influence of an electric potential. The method can also be used to de-salt samples by using an apparatus that only contains a sample stream positioned between the inner and outer buffer streams.

In a third aspect, the present invention provides use of the apparatus according to the first aspect of the present invention in the separation of at least one compound.

In a fourth aspect, the present invention provides a compound separated by the method according to the second aspect of the present invention.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or

group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Any description of prior art documents herein is not an admission that the documents form part of the common general knowledge of the relevant art in Australia.

In order that the present invention may be more clearly understood preferred forms will be described with reference to the following examples and drawings.

Brief Description of Drawings

Figure 1 shows an exploded view of one form of the apparatus according to the present invention.

Figure 2 shows possible electrode shapes for use in the apparatus according to the present invention.

Figure 3 shows a further example of an apparatus according to the present invention having a number of separation membranes with increasing molecular mass cut-offs.

Figure 4 shows a further example of an apparatus according to the present invention having multiple separation tubes or fibres

Modes for Carrying Out the Invention

In one form, the invention involves a hollow fibre approach to electrophoresis, where one electrode in the shape of a thin wire is located at the centre of a series of membranes, preferably concentric membranes, and a second electrode in the shape of a hollow cylinder is located outside the membranes.

A general example is shown in Figure 1 which uses a central electrode, surrounded by a small pore size electrophoretic membrane (inner containment membrane), a second membrane of a suitable pore size to allow passage of molecules of interest (sieving membrane) and an outside containment membrane. An exterior electrode is placed outside the outer restriction membrane.

These membranes define four streams through which fluid (water, aqueous buffer, organic solvent etc) can be passed, preferably by pumping to control the flow.

The stream or channel outside the outer containment membrane contains an appropriate solvent for the extraction procedure, for example an electrophoresis buffer. This solvent can be selected to control the net charge on a molecule of interest.

The stream or channel between the outside containment and sieving membranes contains the sample from which individual molecules are to be separated.

The stream or channel between the sieving and inner containment membranes would initially be filled with appropriate solvent, and serve as the location to where the molecule of interest would be transferred. This channel is termed the separation stream.

The channel inside the inner containment membrane is filled with a suitable solvent, which may be the same as that in the outside channel, or may be different to create solvent gradients across the electrophoretic device.

Sample containing the compound or molecule of interest is passed through the sample stream at a set flow rate and an electrical potential applied between the inner and outer electrodes, causing the electrophoretic migration of charged molecules to the electrode carrying opposite charge.

Through the correct choice of solvent conditions and molecular weight cutoff/pore size of the sieving and containment membranes, a molecule of interest would be transferred from the sample stream to the product stream.

Some variations on the apparatus include:

a) different electrode shapes, for example a cylindrical electrode, a flat strip electrode, an elliptical electrode, cross shaped or other shapes which allow beneficial manipulation of the electrical field (Figure 2).

b) the sieving membrane could be excluded to create a system with two containment membranes for desalting or dialysis applications where removal of contaminants is required.

c) multiple concentric membranes could be employed to effect simultaneous size based separations, for example concentric membranes with 50, 100, 200 etc molecular weight cut-off values could be employed as illustrated in Figure 3.

d) multiple separation tubes with separation channels each with membrane arrangements in a single separation unit define the arrangement of inner electrode, concentric membranes and outer electrode as a single tube or fibre. Many of these fibres could be packed or bundled together in a single

separation unit, with their external solvent streams shared (Figure 4). The number of tubes in a bundle would be determined by the scale of separation or purification required. This type of arrangement of tubes or fibres in a bundle is seen in tissue culture bioreactors and artificial kidney devices.

e) either an entire separation tube or fibre, or a single membrane within a tube or fibre could be spun around an axis defined by the inner electrode at an appropriate speed to create a centrifugal force effect on the samples within the fibre. This centrifugal effect could be used to facilitate separations by including centrifugal force with electrophoretic effects, and could also be used for membrane defouling by spinning contaminants away from the outer faces of the containment or sieving membranes.

f) the centre electrode could be rotated at the centre of the device to create periodic variations in the electric field intensity to facilitate separations which may benefit from such variations.

The uses suggested for this type of electrophoretic separation device include:

Macromolecular separation or purification, micromolecular separation or purification, concentration by transfer from large sample volumes to smaller product volumes, desalting by using a solvent with low ionic strength compared to the sample, concentration using a membrane composition that induces endo-osmotic flow from the sample to the solvent stream, hemodialysis for treatment of blood for the removal of disease related molecules, online extraction of biological products expressed in tissue culture/bioreactor systems, culture of cells within the hollow fibre system, with the option of periodic product extraction and culture medium renewal/refreshment, use of immobilised affinity ligands attached the membranes for procedures requiring a combination of affinity and electrophoretic separations, and use of either free of immobilised enzymes or other catalysts in the sample stream to allow extraction of the reaction product as it is produced catalytically

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this twenty-first day of December 2000

Gradipore Limited
Patent Attorneys for the Applicant:

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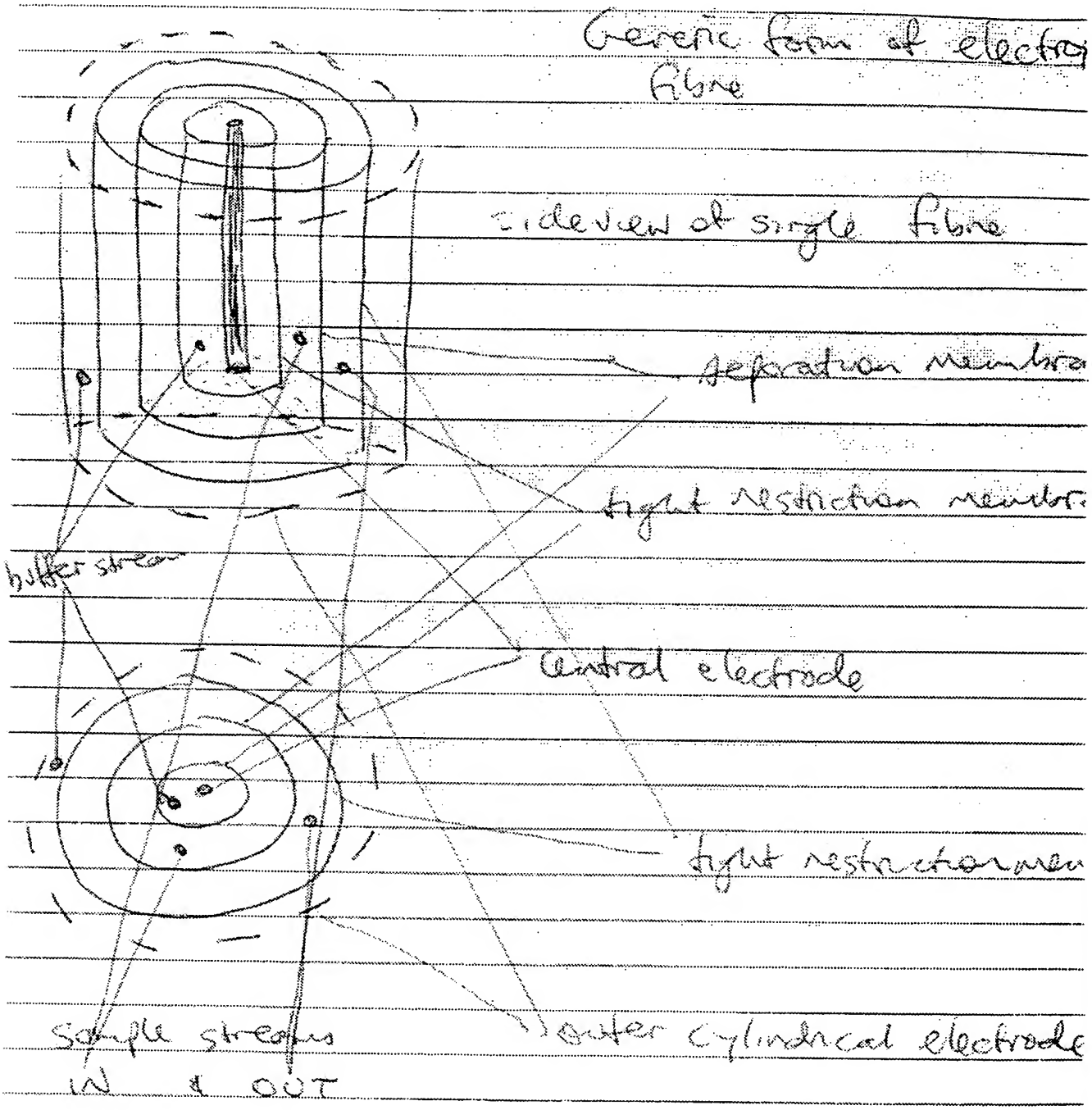

Generic form of electro
fibre
Sideview of single fibre
separation membra
tight restriction membra
buffer stream
central electrode
tight restriction mem
sample streams
IN & OUT
outer cylindrical electrode

Figure 1.

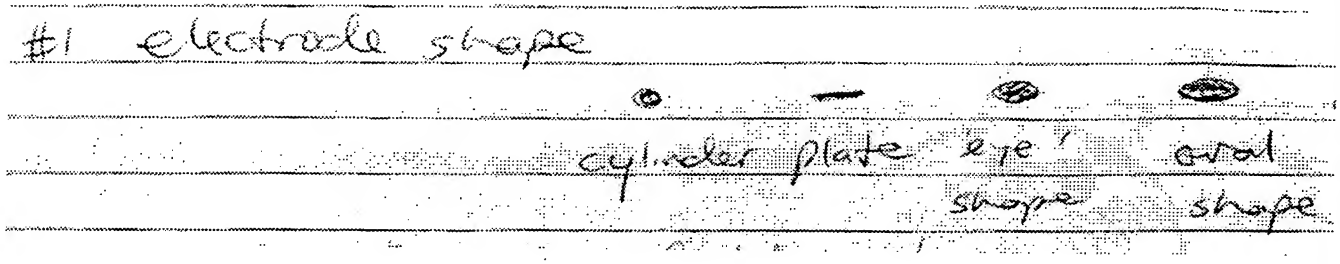


Figure 2.

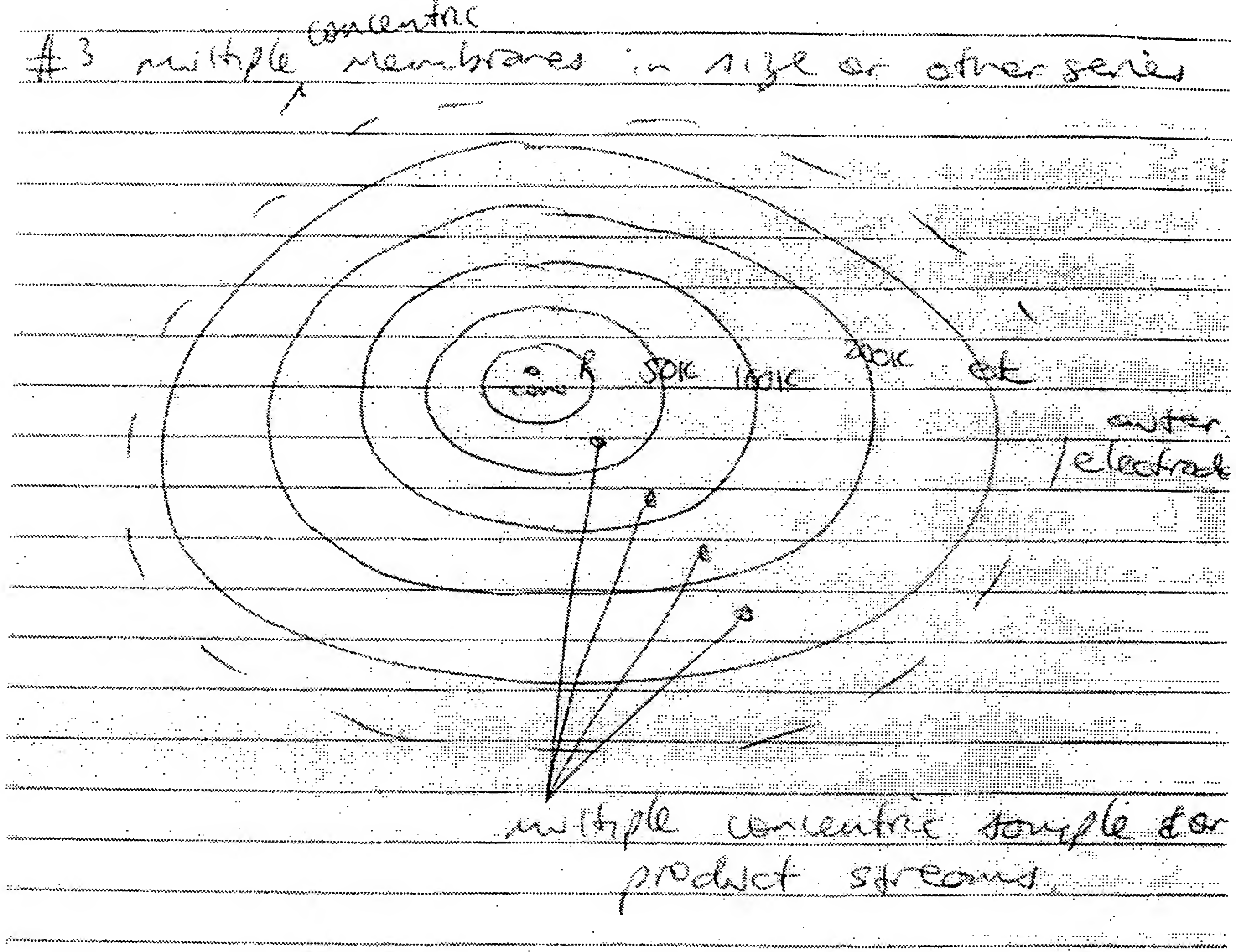
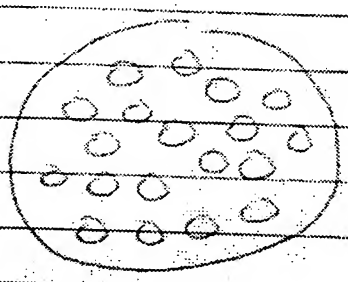

#3 multiple concentric membranes in size or other series
core
R
50K
100K
200K
etc
outer /electrode
multiple concentric sample or product streams

Figure 3.

#4 multiple concentric fibres bundled into one module with shared outside buffer environment



in the same manner as individual fibres are bundled in a bioreactor or artificial kidney.

Figure 4.